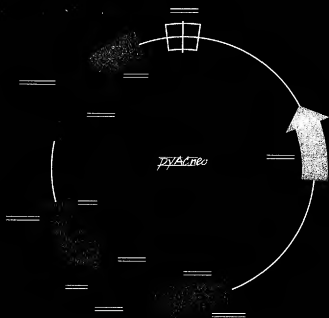


C L O N T E C H

TOOLS FOR THE MOLECULAR BIOLOGIST 91/92



Kb

690

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3 ± 0.01 cm

VECTORS

PROKARYOTIC EXPRESSION VECTORS

pPROK-1

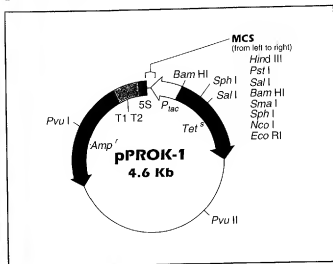


Figure 12.1 Expression vector pPROK-1

pPROK-C

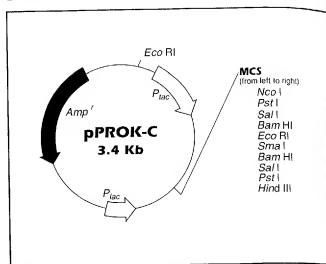


Figure 12.2 Expression vector pPROK-C

MCS

5'...TGT TGA CAA TTA ATC ATC GGC TCG TAT AAT GTG TGG AAT TGT GAG CGG

RBS
ATA ACA ATT TCA CAG ACG AAA CAG AAT TCA
TGC CAT GGC ATG CCC GGG GAT CCG TCG ACC TGC AGC CAA GCT T .3'
Nco I Sma I Sal I Hind III

MCS

CCATGGCTGCAGGTGCGACGGATCC
Pst I Sal I
GGGAATTCCTCCGGGATCGTCCGACCTGCAGCCAAAGCTT
Nco I Sma I Bam HI Pst I Hind III

Product	Size	Cat. #
pPROK-1	25 µg	6002-1

Description

The prokaryotic expression vector pPROK-1 contains *tac*, a strong promoter capable of induction by IPTG in *E. coli* strain JM109. Downstream of the Multiple Cloning Site (MCS) is the strong T1 T2 terminator from the *E. coli rmb* gene preventing unstable replication thus allowing efficient transcript termination. The plasmid mediates both ampicillin and tetracycline resistance.

pPROK-1 has a ribosomal binding site and an ATG start codon positioned immediately upstream of the cloning site for expression of gene fragments which lack these functional units.

Unique cloning sites within the MCS region
Eco RI, Nco I, Sma I, Pst I, Hind III

Vector Size 4.6 Kb

References

1. Brosius, J. (1984) *Gene* 27:151.
2. Brosius, J. (1984) *Gene* 27:161.
3. Amann, E. & Brosius, J. (1985) *Gene* 40:183.

Product	Size	Cat. #
pPROK-C	10 µg	6000-1

Description

pPROK-C is a high efficiency prokaryotic cDNA expression vector. pPROK-C is especially suited for the construction of cDNA expression libraries. These libraries may be screened with both antibody and synthetic oligomer probes. pPROK-C can be used in a wide range of *E. coli* hosts. This vector contains diametrically opposed *lacZ* and *tac* promoters on either side of the Multiple Cloning Site (MCS). Both strands of the inserted DNA can be transcribed and translated, thus cDNA inserted into this vector has twice the probability of being expressed. pPROK-C carries its own ribosomal binding site and initiation ATG start codon.

Unique cloning sites within the MCS region
Sma I, Nco I, Hind III

Vector Size 3.4

pEX1, pEX2, pEX3

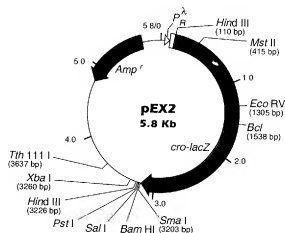


Figure 12.3 Expression vector pEX 2

pEX plasmids polylinker sequences

	Sma I	Bam HI	Sal I	Pst I	Hind III	
pEX1	GCC CGG GGA TCC GTC GAC CTG CAG CCA AGC TTG CTG ATT GAT TGA					
	Ala Arg Gly Ser Val Asp Leu Gln Pro Ser Leu Leu Ile Asp *					
	Eco RI	Sma I Bam HI	Sal I	Pst I	Hind III	
pEX2	GAA TTC CCG GGG ATC CGT CGA CCT GCA GCC AAG CTT GCT GAT TGA					
	Glu Phe Pro Gly Ile Arg Arg Pro Ala Ala Lys Leu Ala Asp *					
		Sma I Bam HI	Sal I	Pst I	Hind III	
pEX3	GAA TTA ATT CCC GGG GAT CCG TCG ACC TGC AGC CAA GCT TGC TGA					
	Glu Leu Ile Pro Gly Asp Pro Ser Thr Cys Ser Gln Ala Cys *					

Description

The pEX family of vectors allow expression of β -galactosidase fusion proteins in *E. coli*. Thermo-inducible expression is driven by the lambda P_R promoter when grown in a suitable *E. coli* host strain carrying the temperature-sensitive repressor cI857 (*E. coli* strain N4830-1). The lambda *cro* gene supplies the ATG start codon. Easy purification of β -galactosidase fusion proteins may be performed on an immunoaffinity column. The *Eco* RI site of pEX2 was 1) treated with SI nuclease to create pEX1, and 2) filled in with DNA polymerase to create pEX3. The three pEX expression vectors, pEX1, pEX2, pEX3 differ only in their reading frames.

Unique cloning sites

Pst I, Sal I, Bam HI, Sma I, Eco RI (pEX1 only)

Vector Size

5.8 Kb

References

1. Stanley, K. K. & Luzzio, J. P. (1984) *EMBO J.* 3:1429.
2. Zabeau, M. & Stanley, K. K. (1982) *EMBO J.* 1:1217.
3. Sugimoto, K., et al. (1977) *J. Mol. Biol.* 111:487.

Product

Size

Cat.

pEX 1	25 μ g	6150-1
pEX 2	25 μ g	6151-1
pEX 3	25 μ g	6152-1

12

Vectors

VECTORS

pKK233-2

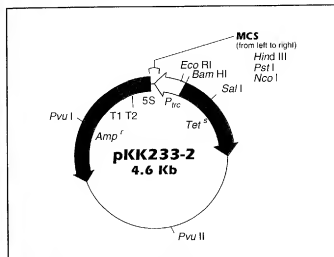


Figure 12.4 Expression vector pKK233-2

MCS

5' -35 -10
 TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGACGGA
 TAACAATTTACACAGGAACAGACCATGGCTGCAGCCAAAGCTTG
 GCTGTTTGGC-3'
 RBS Pst I Nco I Hind III
 rrnB

Product	Size	Cat. #
pKK233-2	25 µg	6003-1

Description

pKK233-2 is designed for high-level expression of cloned genes and cDNAs in *E. coli* strains which are *lacI*^q (e.g., JM105). pKK233-2 features the strong IPTG-inducible *P_{trc}* (trp/lac fusion) promoter [17 base pair spacing between the trp-35 region and the lac UV5 -10 region], the *lacZ* ribosome binding site, and an ATG initiation codon accessible by cleavage at the unique *Nco* I cloning site. The *E. coli* *rrnB* (5S rRNA) T1 and T2 transcription terminators are downstream of the Multiple Cloning Site to prevent read-through and overexpression of the *amp* gene.

Unique cloning sites

Nco I, *Pst* I, *Hind* III

Vector Size	4.6 Kb
-------------	--------

References

- Amann, E. & Brosius, J. (1985) *Gene* 40:183.
- Straus, D. & Gilbert, W. (1985) *Proc. Natl. Acad. Sci. USA* 82:2014
- Shimizu, Y., et al. (1988) *Gene* 65:141.
- Kozak, M. (1983) *Microbiol. Rev.* 47:1.

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pKK338-1

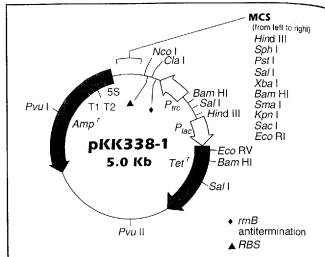


Figure 12.5 Expression vector pKK338-1

Product	Size	Cat. #
pKK338-1	25 µg	6155-1

Description

pKK338-1 is an "ATG" vector containing a strong trc promoter, and a slightly weaker *lacUV5** promoter for high-level expression of cloned proteins. pKK338-1 contains *rrnB* anti-termination sequences downstream of the trc promoter, a synthetic Ribosome Binding Site (RBS) followed by a unique *Nco* I site, the pUC18 polylinker region followed by the 5S rRNA gene, and T1 and T2 transcription terminators from *rrnB*.

* The *lacUV5* promoter does not contain the operator sequence and is not intended for expression.

Unique cloning sites

Nco I, *Eco* RI, *Sac* I, *Kpn* I, *Sma* I, *Xba* I, *Pst* I, *Sph* I

Vector Size	5.0 Kb
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Reference

- Vector: A survey of Molecular Cloning Vectors and their uses, edited by Rodriguez, R.L., and Denhardt, D.T., pp. 213-221 (1988).

pKC30

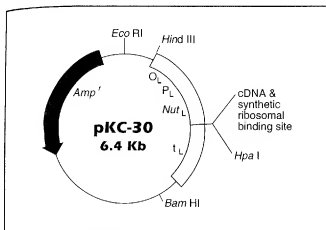


Figure 12.6 Expression vector pKC30

Product	Size	Cat. #
pKC30	25 µg	6103-1

pKT279, pKT280, pKT287

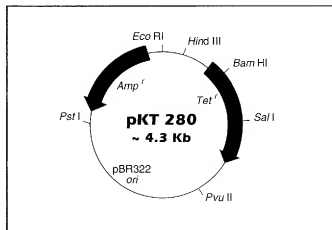


Figure 12.7 Expression/secretion vector pKT280

Product	Size	Cat. #
pKT279	25 µg	6121-1
pKT280	25 µg	6106-1
pKT287	25 µg	6122-1

Description

pKC30 is a thermoinducible expression vector designed for over-expression of eukaryotic cDNA containing its own translational regulatory information [e.g. RBS and ATG]. pKC30 is a pBR322 derivative with a 2.4 Kb *Hind* III-*Bam* HI restriction fragment from λ phage containing the operator (O_L), promoter (P_L) and N recognition site (Nut L). A unique *Hpa* I restriction site is located within the N gene coding region resulting in an N fusion protein upon cloning at this site. The cDNA and ribosome binding site are located within the *Hpa* I site of the N gene region. The P_L promoter is repressed at 30°C and thermoinduced at 42°C.

Unique cloning site

Hpa I

Vector Size

6442 bp

Reference

- Ho, Y. S., et al. (1982) *J. Biol. Chem.* 257:9128.

Description

The three pKT vectors, pKT279, pKT280, and pKT287 are designed for the secretion of cloned gene products into the *E. coli* periplasm. The unique *Pst* I cloning site allows fusion of insert DNA to the promoter and signal peptide sequence of the *E. coli* β -lactamase gene. Tetracycline resistance is available for the amplification and selection of clones in *E. coli*. The three pKT secretion vectors pKT279, pKT280, and pKT287 differ only in their reading frames.

Unique cloning site

Pst I

Vector Size

4.3 Kb

Reference

- Talmadge, K., Stahl, S. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* 77:3369.

pKT279, 280, 287 Secretion Vectors

pKT 279
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCG TTT TTT
GCG GCA TTT TGC CTT CDT GTT TTT GCT CAC CCG **TGC AG**
Pst I

pKT 280
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCG TTT TTT
GCG GCA TTT TGC CTT CDT GTT TTT GCT CAC CCG **CTG CAG**
Pst I

pKT 287
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCG TTT TTT
GCG GCA TTT TGC CTT CDT GTT TTT GCT CAC CCA GAA ACG
GCT GCA G
Pst I

* Sequence following signal peptide sequence is in bold.

VECTORS

pT3/T7-1

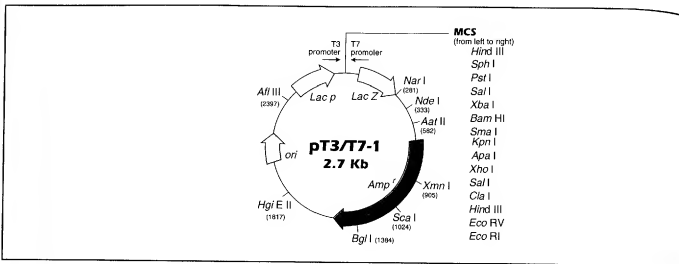
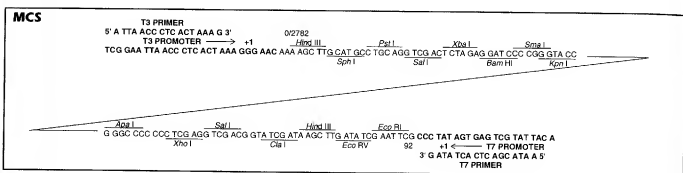


Figure 12.8 Expression vector pT3/T7-1



Description

pT3/T7-1 is a prokaryotic expression vectors containing diametrically opposed T3 and T7 promoters on either side of the the Multiple Cloning Site. This allows for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies, while wild-type colonies will appear as blue colonies, if grown in a suitable bacterial host strain on medium containing X-gal, a chromogenic compound, and IPTG. Antibody screening may be used for detection of β -galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7-1 is derived from pUC19 and is identical to pT3/T7-2 except in the composition of the Multiple Cloning Sites.

Multiple cloning sites

Apa I, Bam HI, Cla I, Eco RI, Eco RV, Hind III, Kpn I, Pst I, Sal I, Sma I, Sph I, Xba I, Xho I

Vector Size

2.7 Kb

References

- Yanisch-Perron, *et al.* (1985) *Gene* 33:109.
- Melton, D.A., *et al.* (1984) *Nucleic Acids Res.* 12:7035.

Product

pT3/T7-1

Size

25 μ g

Cat.

6112-1

pT3/T7-2

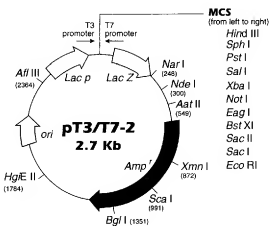
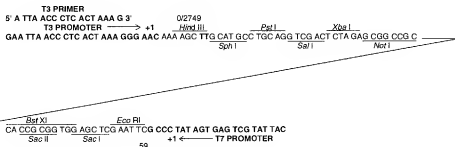


Figure 12. 9 Expression vector pT3/T7-2

MCS



Description

pT3/T7-2 is a prokaryotic expression vectors containing diametrically opposed T3 and T7 promoters on either side of the Multiple Cloning Site. This allows for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies, while wild-type colonies will appear as blue colonies, if grown in a suitable bacterial host strain on medium containing X-gal, a chromogenic compound, and IPTG. Antibody screening may be used for detection of β -galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7-2 is derived from pUC19 and is identical to pT3/T7-1 except in the composition of the Multiple Cloning Sites.

Multiple cloning sites

Bst XI, Eag I, Eco RI, Hind III, Not I, Pst I, Sac I, Sac II, Sal I, Sph I, Xba I

Vector Size

2.7 Kb

References

1. Yanisch-Perron, *et al.* (1985) *Gene* 33:109.
2. Melton, D.A., *et al.* (1984) *Nucleic Acids Res.* 12:7035.

Product

pT3/T7-2

Size

25 μ g

Cat.

6113-1

VECTORS

pT3/T7-3

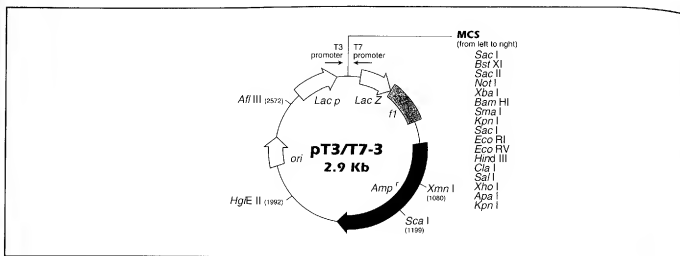
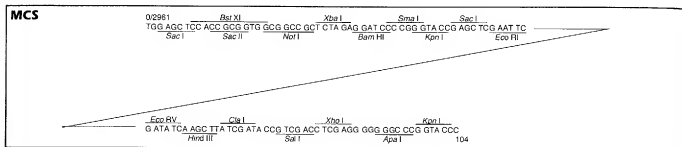


Figure 12.10 Expression vector pT3/T7-3



Description

pT3/T7-3 is a prokaryotic expression vector carrying the f1 intergenic region from M13, allowing rescue of single-stranded clones with aid of a helper phage. pT3/T7-3 contains diametrically opposed T3 and T7 promoters on either side of the Multiple Cloning Site for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies while wild-type colonies will appear blue if grown in a suitable bacterial host strain on medium containing X-gal and IPTG.

Antibody screening may be used for detection of β -galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7 phagemid is a pUC19 derivative.

Multiple cloning sites

Sac I, Sac II, Bst XI, Not I, Xba I, Bam HI, Sma I, Kpn I, Eco RI, Eco RV, Hind III, Cla I, Sal I, Xho I, Apa I

Vector Size

2.9 Kb

Product

pT3/T7-3

Size

25 μ g

Cat.

6114-1

pT3/T7-LUC

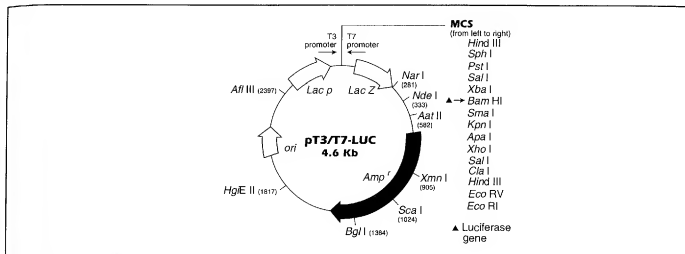
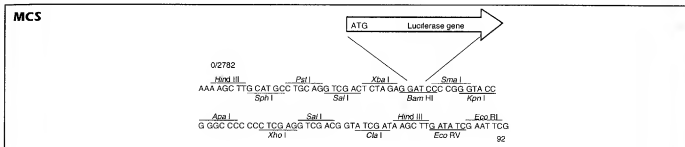


Figure 12.11 Expression vector pT3/T7-LUC



Description

pT3/T7-LUC is a prokaryotic expression vector derived from pT3/T7-1 and containing a luciferase reporter gene useful for testing promoter sequences upstream of the *Bam* HI site. pT3/T7-LUC offers one of the most sensitive reporter genes controlled by the *lacZ* promoter sequences. The 1.9 Kb luciferase coding region comes from the firefly luciferase gene. The *luc* gene may be excised by *Bam* HI, *Hind* III or *Sal* I digestion. In addition the T3 and T7 promoters may be used to generate single-stranded RNA probes by *in vitro* transcription.

Luciferase is an enzyme that catalyzes the light-producing chemical reaction of certain bioluminescent organisms. In the presence of luciferin and ATP, the enzyme-bound luciferyl-adenylate complex is formed, which is followed by oxidative decarboxylation. The reaction products are CO₂, oxyluciferin, AMP, and light. The light emission may be measured spectrophotometrically or observed on an x-ray film. The luciferase bioassay has been shown to be many times more sensitive than the β-galactosidase or other reporter gene assays. This makes the luciferase gene an important tool for expression studies in micro-organisms, plants, and animals.

Product	Size	Cat. #
pT3/T7-LUC	25 µg	6170-1

Unique sites within the MCS region

Apa I, *Kpn* I, *Pst* I, *Sma* I, *Xba* I, *Xho* I

Vector Size	4.6 Kb
-------------	--------

References

1. DeWet, J. R., et al. (1987) *Mol. Cell. Biol.* 7:725.
2. Ow, D. W., et al. (1986) *Science* 234:856.
3. Rodríguez, J.F., et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:1667.

VECTORS

EUKARYOTIC EXPRESSION VECTORS

pEUK-C1

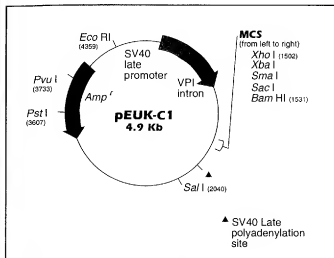
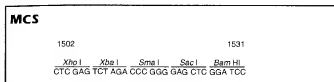


Figure 12.12 Expression vector pEUK-C1



Product	Size	Cat. #
pEUK-C1	25 µg	6107-1

Description

pEUK-C1 is a mammalian expression vector designed for high-level expression of eukaryotic genes in COS cells due to the absence of pBR322 "Poison sequences" thus allowing a higher copy number. The expression of cloned genes is under the control of the SV40 late promoter. pEUK-C1 contains SV40 termination, splicing, and polyadenylation elements as well as SV40 and pBR322 origins of replication.

Unique cloning sites within the MCS region

Xho I, Xba I, Sma I, Sac I, Bam HI

Vector Size

4.9 Kb

References

- Templeton, D. & Eckhart, N. (1984) *Mol. Cell. Biol.* 4:817.
- Lusky, M. & Botchan, M. (1981) *Nature* 293:79.

pEUK-C2

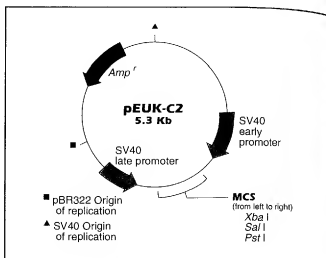


Figure 12.13 Expression vector pEUK-C2

Product	Size	Cat. #
pEUK-C2	10 µg	6001-1

Description

pEUK-C2 is a high efficiency eukaryotic cDNA expression vector. It is distinguished by two diametrically opposed SV40 (Early and Late) promoters. Both strands of the inserted DNA can be transcribed and translated, thus any cDNA inserted into this vector has twice the probability of being expressed. The unique Pst I site may be used to construct cDNA libraries. pEUK-C2 contains the SV40 and pBR322 origins of replication. High level expression of eukaryotic genes in COS cells is due to the absence of pBR322 "Poison sequences." The *E. coli* gene for ampicillin resistance makes this vector easy to amplify in *E. coli*.

Unique cloning sites within the MCS region

Pst I, Sal I, Xba I

Vector Size

5.3 Kb

Reference

- Davis, E., Harpaz, N., Johnson, E. (1990) *JBC* 265 (38):22153.

YEAST CLONING & EXPRESSION VECTORS

Lambda Max1 & pYEura3

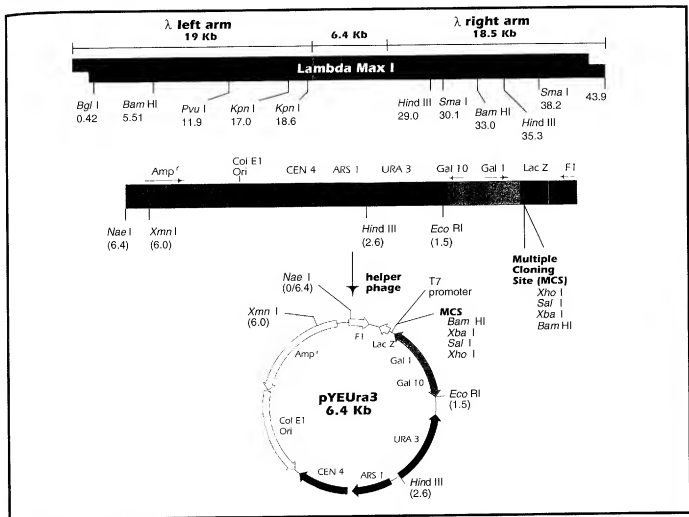


Figure 12.14 Schematic diagram of Lambda Max1 and pYEura3. pYEura3 sequences can be excised from Lambda Max1 and converted into the plasmid by helper phage.

Recent molecular analysis of genes reveals a striking similarity in both structure and function between mammalian and yeast genes. Although molecular cloning techniques have elucidated gene structures at an unprecedented rapid pace, the functions of many of these genes remain elusive. Studies are now being conducted to determine gene function in yeast systems, and what is learned from these studies in yeast may eventually be applied to mammalian systems. A number of yeast vectors have been designed and constructed for cloning cDNAs so that their functions may be identified in yeast cells. However, the cloning efficiency in plasmid-type yeast vectors is generally low and this factor seriously limits their utility for these studies. Therefore, we have developed Lambda Max1 to enable both efficient cloning and yeast expression.

The design of Lambda Max1 incorporates the properties of lambda phage that make it highly efficient in cloning cDNA, as λ gt10 and λ gt11 can be, together with several yeast elements that enable propagation and expression of the cloned cDNA in yeast cells.

The yeast elements include the inducible GAL1 and GAL10 promoters as well as URA3, CEN4, and ARS1. These yeast elements, together with the multiple cloning site, can be excised and converted into the plasmid pYEura3 by helper phage for transformation and expression of the cloned cDNA in yeast.

VECTORS

Lambda Max 1™

- cDNA cloning vector for yeast expression studies

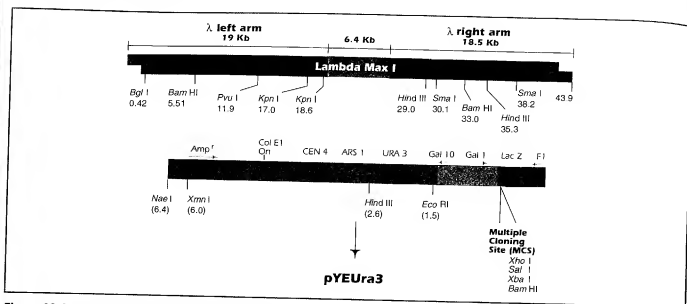


Figure 12.15 Yeast cDNA cloning vector Lambda Max 1.

Product	Size	Cat. #
Lambda Max 1	20 µg	6192-1
Lambda Max 1 arms (Xba I/Xho I)	10 µg	6193-1
Lambda Max 1 arms (Eco RI-digested)	10 µg	6194-1

Description

A unique Yeast cloning vector designed for high-efficiency cloning of cDNA and restriction enzyme digests. Plasmid with the cDNA sequence can be excised with helper phage and converted into the pYEura3 plasmid by helper phage, thus circumventing subcloning steps. Plasmid cDNA is then ready for mapping and sequencing. Lambda Max 1 allows generation of cDNA libraries of high complexity (>10⁶ clones), and clones cDNA up to 7.0 Kb in length.

Lambda Max 1 enables directional cloning of cDNA to enhance yeast expression and is ideal for expression studies of mammalian and other eukaryotic genes in yeast, to identify human analogs of yeast genes, and to find a yeast gene equivalent to your mammalian gene. The Lambda Max 1 arms are also available separately, digested with either Xba I/Xho I or with Eco RI. Complete protocols and helper phage are included with each product.

Unique cloning sites

Xba I, Xho I, Bam HI, Sal I

Vector size

44 Kb

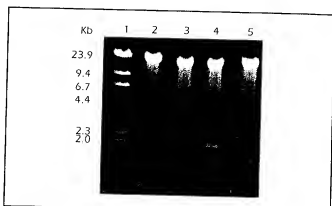


Figure 12.16 Human HeLa cDNA library in Lambda Max 1. Four clones (Lanes 2-5) were randomly picked and their inserts excised by Xba I and Xho I digestion. Lane 1: Lambda DNA/Hind III size marker.

pYEura3™

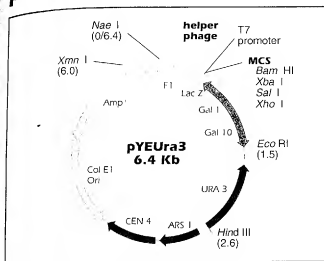


Figure 12.17 Yeast expression vector pYEura3.

Product	Size	Cat. #
pYEura3	20 µg	6195-1

Description

A yeast expression vector with the URA3 marker. pYEura3 contains the strong Gal I and Gal 10 promoters and other elements that can propagate and express cDNA in yeast cells. Also contains selection markers for propagation of plasmids in *E. coli*. Routinely yields frequencies of 10^5 yeast transformants/µg. A complete protocol is included.

Unique cloning sites

Bam HI, Xba I, Sal I, Xho I

Vector size

6.4 Kb

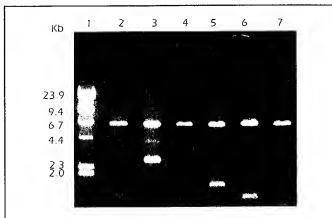


Figure 12.18 Human HeLa cDNA Library in pYEura3. Six colonies (Lanes 2-7) were randomly picked and their inserts excised by Xba I/Xho I. The Human HeLa cDNA Library was generated by converting the library (Lambda Max 1) into the plasmid pYEura3 using a helper phage and then transforming the plasmids into bacteria. Lane 1: Lambda DNA/Hind III size marker.



Figure 12.19 Yeast transformants. The entire human HeLa cell library in pYEura3 transformed into competent yeast cells. Approximately 10^5 transformants were scored per µg of recombinant DNA used.

VECTORS

pG1

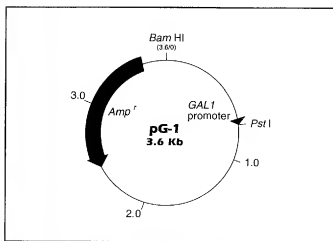


Figure 12.20 Yeast cDNA cloning vector pG1

Product	Size	Cat. #
pG1	25 µg	6135-1

Description

pG1 is a yeast cDNA cloning vector used as the Okayama-Berg "adaptor" for the construction of cDNA expression libraries in *Saccharomyces cerevisiae*. The galactose-inducible GAL1 promoter is on a 0.8 Kb *Bam* HI-*Pst* I fragment upstream of the *Pst* I cloning site. pG1 is used in tandem with pTRP56 in *S. cerevisiae* strain SHY2 for high efficiency cloning and expression of full-length cDNAs in yeast. The ampicillin resistance gene is present for selection in *E. coli*.

Unique cloning site

Pst I

Vector Size	3.6 Kb
-------------	--------

References

1. Muyajma, A., et al. (1984) *Nucleic Acids Res.* 12:6397.
2. Goff, C. G., et al. (1984) *Gene* 27:35.

pTRP56

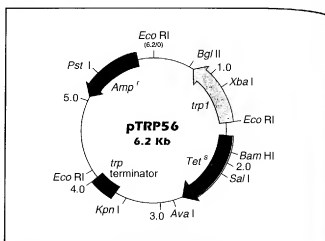


Figure 12.21 Yeast expression vector pTRP56

Product	Size	Cat. #
pTRP56	25 µg	6130-1

Description

pTRP56 is a cDNA yeast cloning vector designed to be used in conjunction with pG1 as the Okayama-Berg "primer" for the construction of cDNA expression libraries in *Saccharomyces cerevisiae*. pTRP56 carries the ARS yeast element allowing autonomous replication in *S. cerevisiae*. The TRP1 selection marker is used for selection in *S. cerevisiae* strain SHY2. Transformants are selected by complementation of Trp¹ strains on media which lack tryptophan. The resulting cDNA construct can be propagated in either *Saccharomyces* or *E. coli*. Ampicillin resistance is present for selection in *E. coli*.

Vector Size	6.2 Kb
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References

1. Muyajma, A., et al. (1984) *Nucleic Acids Res.* 12:6397.
2. Goff, C. G., et al. (1984) *Gene* 27:35.

pYACneo

Yeast artificial chromosome cloning vector

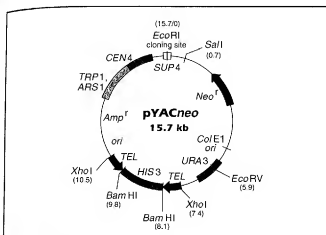


Figure 12.22

Product	Size	Cat. #
pYACneo	20 µg	6190-1
	120 µg	6190-2
pYACneo arms	20 µg	6191-1
	100 µg	6191-2

Description

Yeast artificial chromosome [YAC] cloning vector for use in cloning large DNA fragments up to 1.0 Megabase to facilitate genome mapping and sequencing projects.

pYACneo contains *Xho*I sites for plasmid rescue of both ends of the insert, and the neomycin resistance gene to allow transfer of a positive YAC clone to mammalian cells. Upon insertion of *Eco*RI digested fragments, the *sup4-O* yeast nonsense suppressor becomes inactivated; this reduces the background of non-recombinants and allows for their visual detection as light-pink rather than red.

The pYAC neo arms, prepared by digestion of pYACneo with *Eco*RI and *Bam*HI followed by dephosphorylation, are also available separately. Each product includes *Eco*RI control DNA insert, a complete protocol for vector preparation, and an annotated bibliography for developing cloning strategies.

Vector size 15.7 Kb

References

- Burke, D.T. *et al.* (1987) *Science* **236**:806.
- Albertson, *et al.* (1990) *Proc. Natl. Acad. Sci.* **87**:4256.
- Anand, *et al.* (1990) *Nucleic Acids Res.* **18**:1951.

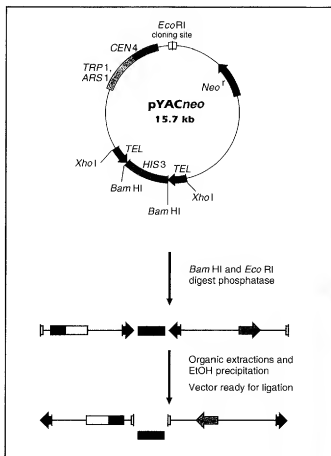


Figure 12.23

12

Vectors

VECTORS

MAMMALIAN EXPRESSION VECTORS

pMAM

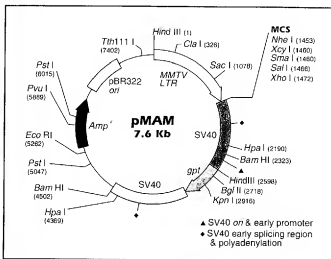
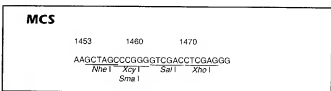


Figure 12.24 Expression vector pMAM



Product	Size	Cat. #
pMAM	20 µg	6100-1

Description

pMAM is the parental plasmid of the pMAM mammalian expression vector family. pMAM offers dexamethasone-inducible expression driven by the MMTV-LTR promoter and contains the *E. coli* gpt gene, which allows selection of transformants in HAT medium, and the SV40 ori, splicing, and polyadenylation elements.

Unique cloning sites within the MCS region

Nhe I, Xcy I, Sma I, Sal I, Xho I

Vector Size

7.6 Kb

pMAMneo

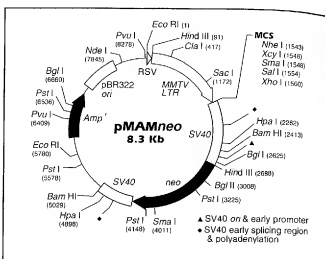
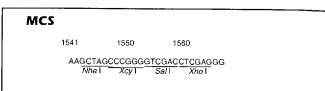


Figure 12.25 Expression vector pMAMneo



Product	Size	Cat. #
pMAMneo	25 µg	6104-1

Description

pMAMneo is a mammalian expression vector designed for high level expression of eukaryotic genes. pMAMneo contains the RSV-LTR enhancer linked to the dexamethasone-inducible MMTV-LTR promoter. This construction yields controllable yet high level expression of cloned genes in the presence of dexamethasone. The SV40 splicing and polyadenylation sites provide RNA processing in mammalian cells while the *E. coli* neo gene, driven by the SV40 early promoter allows selection of transformants growing in media containing G418 antibiotics.

Unique cloning sites within the MCS region

Nhe I, Sal I, Xho I

Vector Size

8.3 Kb

References

- Lee, F., et al. (1981) *Nature* 294:228.
- Sardet, C., Franchi, A. & Pouyssegur, J. (1989) *Cell* 56:271.

pMAMneo-BLUE

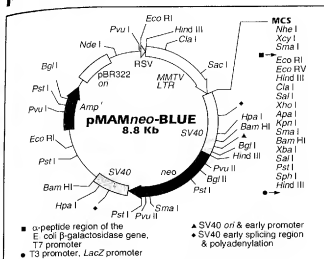


Figure 12.26 Expression vector pMAMneo-BLUE

pMAMneo-CAT

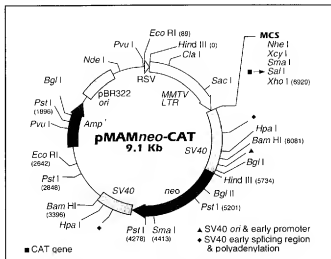
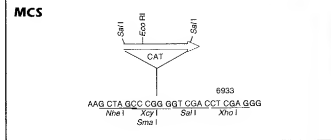
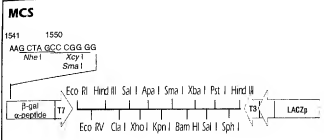


Figure 12.27 Expression vector pMAMneo-CAT



Product	Size	Cat. #
pMAMneo-BLUE	25 µg	6105-1

Product	Size	Cat. #
pMAMneo-CAT	25 µg	6141-1

Description

pMAMneo-BLUE is a mammalian expression vector derived from pMAMneo by the insertion of a 540 bp fragment containing the *lacZ* promoter, the α -peptide regions of the β -galactosidase gene, the T3 and T7 RNA polymerase promoters, and a polylinker region cloned into the multiple cloning site of pMAMneo. Recombinant transformants in *E. coli* may be immuno- or blue/white colony screened. Cloned gene expression is regulated by induction with dexamethasone. The T3 and T7 promoters provide *in vitro* transcription of cloned gene fragments.

Unique cloning sites within the MCS region

Nhe I, Eco RV, Xho I, Kpn I, Xba I

Vector Size

8.8 Kb

Description

pMAMneo-CAT is a eukaryotic expression vector and by virtue of an easily-detected chloramphenicol acetyltransferase (CAT) activity is useful in three separate ways: (1) as a positive transfection control; (2) for testing the function of cloned regulatory sequences; and (3) as a method of providing fusion polypeptides for the stabilization of cloned gene products. The CAT gene found in pMAMneo-CAT is from the *E. coli* Trn9 transposon. The RSV-LTR enhancer is linked to the dexamethasone-inducible MMTV-LTR promoter for controlled yet high-level expression of CAT. pMAMneo-CAT is equipped with the SV40 ori, splicing, and polyadenylation sites.

Unique cloning sites within the MCS region

Nhe I, Xho I

Vector Size

9.1 Kb

References

1. Close, T.J. & Rodriguez, R. (1982) *Gene* 20:305.
2. Lee, F., et al. (1981) *Nature* 294:228.

VECTORS

PMAMneo-LUC

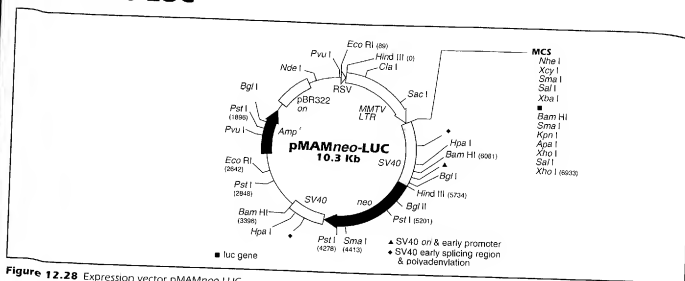
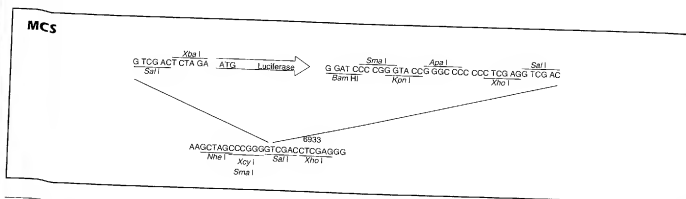


Figure 12.28 Expression vector pMAMneo-LUC



Description

pMAMneo-LUC is a mammalian expression vector derived from pMAMneo that contains the firefly luciferase reporter gene. pMAMneo-LUC offers one of the most sensitive reporter genes available. The 1.9 Kb luciferase coding region of the firefly luciferase gene was cloned into the pMAMneo Sal I site. pMAMneo-LUC contains SV40 ori, splicing, and polyadenylation elements. In addition, pMAMneo-LUC features stabilization of cloned gene products by fusion to luciferase. The pBR322 ori and amp genes allow easy propagation and amplification in *E. coli*.

Luciferase is an enzyme that catalyzes the light-producing chemical reaction of some bioluminescent organisms. In the presence of luciferin and ATP, the enzyme-bound luciferin-adenylate complex is formed, which is followed by oxidative decarboxylation. The reaction products are CO₂, oxyluciferin, AMP, and light. The light emission may be measured spectrophotometrically or observed on an x-ray film. The luciferase bioassay has been shown to be about 1000 times more sensitive than the β -galactosidase or other reporter gene assays. The luciferase gene is therefore a useful tool for expression studies in microorganisms, plants, and animals.

Product

pMAMneo-LUC

Size

25 μ g

Cat.

6171-1

Unique cloning sites within the MCS region

Xba I, Kpn I

Vector Size

10.3 Kb

References

1. DeWet, J.R., et al. (1987) *Mol. Cell. Biol.* 7:725.
2. Ow, D.W., et al. (1986) *Science* 234:856.
3. Rodriguez, J.F., et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:1667.

pCH110

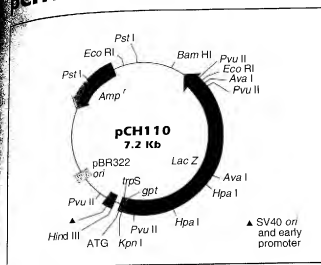


Figure 12.29 Expression vector pCH110

Product	Size	Cat. #
pCH110	25 µg	6101-1

Description

pCH110 is a transient expression vector containing a functional *lacZ* gene expressed from either the SV40 early promoter or from the *E. coli* *gpt* promoter. pCH110 is especially suited for transient expression of β -galactosidase in COS cells. The β -gal level can be monitored for studying mammalian cell expression or as an indicator of the effects of altered cell culture conditions on gene expression. pCH110 contains both the SV40 and pBR322 origins of replication, the *amp* gene for selection in *E. coli*, and the SV40 early promoter fused to the *E. coli* *lacZ* gene. pCH110 may be used as a positive control to normalize cloned gene expression in COS cells, as well as for the determination of recombinant transformation efficiency by β -gal activity assays. When pCH110 is co-transfected with a second plasmid, it can serve as an internal marker for monitoring and normalizing transient and stable expression between different experiments in eukaryotic cells. pCH110 allows detection of recombinant expression by an easy colorimetric assay for β -galactosidase via fusion of cloned genes to a segment of the *lacZ* gene.

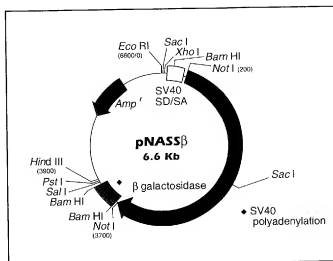
Unique cloning sites

Hind III, Kpn I

Vector Size 7.3 Kb

References

- Hall, C. V., et al. (1983) *J. Mol. Appl. Gen.* 2:101.
- Miller, J. H. in *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory) (1972).
- Herbomel, P., et al. (1984) *Cell* 39:653.
- Lee, F. et al. (1984) *Nucleic Acids Res.* 12:4191.

pNASS β Figure 12.30 Expression vector pNASS β

Product	Size	Cat. #
pNASS β	20 µg	6175-1

Description

pNASS β is a promoterless mammalian expression vector that allows cloning and testing of promoters using β -galactosidase expression. pNASS β contains the SV40 RNA splice site, a polyadenylation signal, and the *E. coli* β -gal gene. The β -gal gene is located within a pair of *Not* I restriction sites for easy excision and replacement with another gene of interest.

Unique cloning sites

Eco RI, Xho I, Sal I, Pst I, Hind III

Vector Size 6.6 Kb

References

- MacGregor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
- Norton & Coffin (1985) *Mol. and Cell Biol.* 5:281.
- Alam, J. (1990) *Anal. Biochem.* 188:245-254.

VECTORS

pAD β

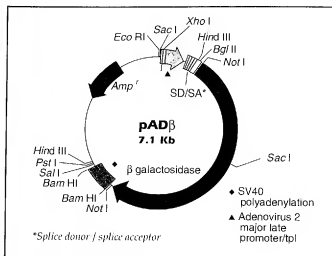


Figure 12.31 Expression vector pAD β

Product	Size	Cat. #
pAD β	25 μ g	6176-1

Description

pAD β is a mammalian expression vector designed for the expression of β -galactosidase in mammalian cells as transcribed by the Adenovirus promoter. pAD β contains an RNA splice site, a polyadenylation signal, and the *E. coli* β -galactosidase gene. The β -gal gene is located within a pair of *Not* I restriction sites allowing easy excision and replacement with another gene of interest. pAD β may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β -gal stain.

Cloning site

Not I

Unique sites

Eco RI, *Pst* I

Vector Size

7.1 Kb

References

- MacGreggor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
- Norton & Coffin (1985) *Mol. and Cell Biol.* 5:281.
- Alam, J. (1990) *Anal. Biochem.* 188:245-254.

pCMV β

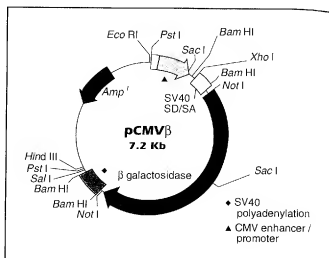


Figure 12.32 Expression vector pCMV β

Product	Size	Cat. #
pCMV β	25 μ g	6177-1

Description

pCMV β is a mammalian expression vector designed for the expression of β -galactosidase in mammalian cells as transcribed by the Cytomegalovirus promoter. pCMV β contains an RNA splice site, a polyadenylation signal and the *E. coli* β -gal gene. The β -gal gene is located within a pair of *Not* I restriction sites for excision and replacement with another gene of interest. pCMV β may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β -gal stain.

Cloning site

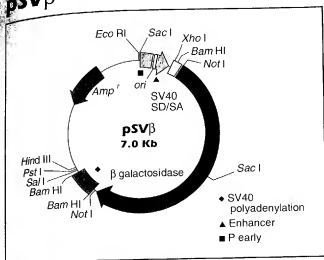
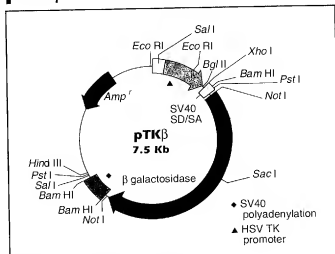
Not I

Vector Size

7.2 Kb

References

- MacGreggor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
- Norton & Coffin (1985) *Mol. and Cell Biol.* 5:281.
- Alam, J. (1990) *Anal. Biochem.* 188:245-254.

pSV β Figure 12.33 Expression vector pSV β pTK β Figure 12.34 Expression vector pTK β

Product	Size	Cat. #
pSV β	25 μ g	6178-1

Description

pSV β is a mammalian expression vector designed for the expression of β -galactosidase in mammalian cells as transcribed by the SV40 promoter. pSV β contains an RNA splice site, a polyadenylation signal, and the *E. coli* β -gal gene. The β -gal gene is located within a pair of *Not* I restriction sites used for easy excision and replacement with another gene of interest. pSV β may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β -gal stain.

Cloning site

Not I

Vector Size

7.0 Kb

References

- MacGregor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
- Norton & Coffin (1985) *Mol. and Cell Biol.* 5:281.
- Alam, J. (1990) *Anal. Biochem.* 188:245-254.

Product	Size	Cat. #
pTK β	25 μ g	6179-1

Description

pTK β is a mammalian expression vector designed for the expression of β -galactosidase in mammalian cells as transcribed by the Thymidine Kinase promoter. pTK β contains an RNA splice site, a polyadenylation signal, and the *E. coli* β -gal gene. The β -gal gene is located within a pair of *Not* I restriction sites used for easy excision and replacement with another gene of interest. pTK β may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β -gal stain.

Cloning site

Not I

Vector Size

7.5 Kb

References

- MacGregor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
- Norton & Coffin (1985) *Mol. and Cell Biol.* 5:281.
- Alam, J. (1990) *Anal. Biochem.* 188:245-254.

12

Vectors

VECTORS

CLONING VECTORS

pUC18, pUC19

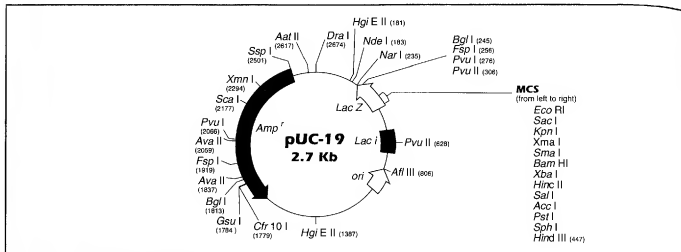


Figure 12.35 Expression vector pUC19

Description

pUC18 and pUC19 are prokaryotic cloning vectors sharing a multiple cloning site with more than 13 unique sites. This Multiple Cloning Site is located within the *lacZ* gene, resulting in the disruption of β -galactosidase activity by cloned inserts and the appearance of white colonies on X-gal + IPTG containing plates. The *E. coli amp* and *ori* elements allow for easy *E. coli* amplification and selection. pUC18 and pUC19 differ in the orientation of the Multiple Cloning Site.

Unique cloning sites within the MCS region

Acc I, Bam HI, Eco RI, Hinc II, Hind III, Kpn I, Pst I, Sac I, Sal I, Sma I, Sph I, Xba I, Xma I

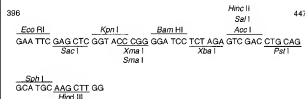
Vector Size

2.7 Kb

References

- Yanisch-Perron, C., et al. (1985) *Gene* 33:103.
- Norlander, J., et al. (1985) *Gene* 26:101.

MCS



Product

Size

Cat.

pUC18	20 µg	6110-1
pUC19	20 µg	6111-1

Lambda BlueMid™

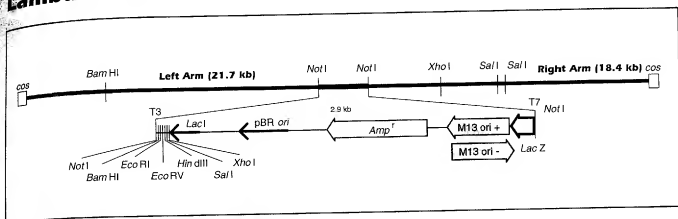


Figure 12.36 cDNA cloning vector Lambda BlueMid

Product	Size	Cat. #
Lambda BlueMid (+)	10 µg	6119-1
Lambda BlueMid (-)	10 µg	6120-1

Description

cDNA cloning vectors for high-efficiency cloning with easy conversion to plasmid. Lambda BlueMid is designed with unique features that facilitate cloning large cDNAs (up to 7.0 Kb) and removal of complete inserts by converting them to plasmids. Thus, no subcloning or helper phage needed for plasmid conversion. Contains T3 and T7 promoters for in vitro transcription and bi-directional restriction mapping, and a M13 origin for easy single-stranded sequencing. Allows directional cloning into *Hind* III and *Eco* RI sites.

Lambda BlueMid is available as the (+) or (-) form, distinguished by the orientation of the M13 ori sequence after digestion with *Eco* RI and *Hind* III and dephosphorylation. A *Hind* III/*Eco* RI cloning linker/adaptor and complete protocol is provided.

Unique cloning sites

Bam HI, *Not* I, *Not* I, *Xho* I, *Sal* I

Vector size

43 Kb

VECTORS

pWE15

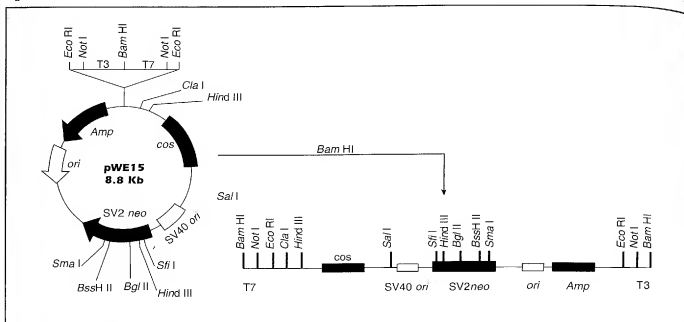


Figure 12.37 Cosmid cloning vector pWE15

Product	Size	Cat. #
pWE15	20 µg	6340-1
	100 µg	6340-2

Description

pWE15 is a cosmid cloning vector designed for rapid restriction mapping of genomic clones. This cloning vector permits the *in vitro* packaging of genomic DNA fragments in the 35-45 Kb range. Nested inside the 8 bp *Not* I sites are the T3 and T7 transcriptional promoters which flank the *Bam* HI cloning site to facilitate generation of end-specific RNA probes for the rapid identification of overlapping fragments (also known as chromosomal walking). Release of intact cloned insert DNA is accomplished by virtue of the rare-cutting *Not* I restriction enzyme flanking the *Bam* HI cloning site. pWE15 contains an SV40 promoter driving the neomycin selection marker for expression in eukaryotic cells. The presence of the ampicillin resistance gene also allows for prokaryotic selection.

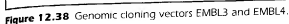
Unique cloning site

Bam HI

Vector Size 8.8 Kb

References

1. Wahl, G., et al. (1987) *Proc. Natl. Acad. Sci. USA* **84**:2160.
2. Lau, Y. F. & Kan, Y. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**:5225.
3. Ish-Horowitz & Burke, J. F. (1981) *Nucleic Acids Res.* **9**:2989.



Description
EMBL-3 and 4 are high-capacity lambda replacement vectors which are particularly useful for the construction of genomic clone banks with partial *Mbo* I digests. Genomic cloning with lambda replacement vectors is more efficient for donor fragment recovery, and recombinant screening than is genomic cloning with cosmid vectors. These cloning vectors are suitable for cloning genomic DNA fragments in the 9.0-22.0 Kb range. Recombinants are easily selected by virtue of their *Spi* phenotype, which allows the phage to be amplified in *E. coli* strain K803 for EMBL-3, and K802 for EMBL-4.

42.2 Kb

1. Frischauf, A. M., et al. (1983) *J. Mol. Biol.* **170**:827.
2. Karn, J. M., et al. (1980) *Proc. Natl. Acad. Sci. USA* **77**:5172.

Vectors

VECTORS

Additional cDNA Cloning Tools

Product	Size	Cat. #
Lambda DNA	500 µg 2000 µg	6250-1 6250-2
Lambda gt10 DNA, uncut	10 µg	6332-1
Lambda gt11 DNA, uncut	10 µg	6333-1
Lambda gt Cloning Linker, <i>Eco</i> RI, dephosphorylated 12mer	2.5 µg	6490-1
Lambda gt10 DNA, <i>Eco</i> RI cut	10 µg	6330-1
Lambda gt11 DNA, <i>Eco</i> RI cut	10 µg	6331-1
pBR322 DNA	20 µg 100 µg	6210-1 6210-2
pBR322; dG-tailed, <i>Pst</i> I cut	5 µg 25 µg	6230-1 6230-2